DEPARTMENT OF PESTICIDE REGULATION

PEST MANAGEMENT GRANTS PROGRAM

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Title: Augmentative Biological Control Using Transplants

Principal Investigator: Charles H. Pickett

Address: CDFA, 3288 Meadowview Rd., Sacramento, CA 95832

Telephone: (916) 262-2053 Fax: (916) 262-2059 e-mail: cpickett@cdfa.ca.gov

Principle Investigator: Greg Simmons

Address: USDA-APHIS PPQ, 355 S. Vineland Rd., Bakersfield, CA 93307

Disclaimer

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Acknowledgements

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Abstract

During the 1990's several new parasitoids were imported for control of the silverleaf whitefly in southern California. Initial studies showed that parasitism of this pest in cantaloupe could be increased through early season releases of *Eretmocerus* spp. A novel technique to increase the efficiency of releases was proposed. The use of transplants, inoculated with recently imported *Eretmocerus* nr. *emiratus* to augment parasitism (banker plants) was explored during three growing seasons, springs 1998 to 2000. Methods were developed to successfully inoculate, then transfer plants into both organic and conventional fields of cantaloupe. Several obstacles to inoculating plants with adequate number of parasitized whiteflies were overcome. In 1998 and 1999 the transplant method (banker plants) was compared to a standard hand release one, and a no-release control in a replicated small plot (1/3 ac) study at an organic farm. Both years, the whitefly numbers were lowest in the plots receiving parasites. However, no consistent, significant differences were detected across both years when comparing insect population means between the two release strategies: hand versus banker plants. Parasitism means were consistently higher in the banker plant plots in 1999, but about the same as in hand release plots in 1998. In 2000 we set up paired comparisons in 7 commercial farms of cantaloupe in Imperial Valley to demonstrate and measure the impact of banker plants on parasite and whitefly populations: 3 organic and four conventional, i.e. imidacloprid for whitefly control. A repeated measures ANOVA, blocking for type of grower (organic vs. conventional) showed that banker plants significantly increased parasitism in study sites over the duration of the study, April through June, 2000 ($p=0.03$). Parasitism was higher in organic fields (seasonal average = 51%) than conventional ones (seasonal average = 10%; Fig. 3). A repeated measures ANOVA showed that banker plants, on average, had no impact on whitefly populations. Plots receiving banker plants, whether organic or conventional, had the same silverleaf whitefly population density from April through June as the paired controls ($\alpha=0.05$). However, some study sites showed a consistent suppression of whiteflies in treated plots over control plots. Differences in parasite numbers and parasitism between the organic and conventional plots suggest that imidacloprid reduced their numbers.

Executive Summary

We report on a novel approach to enhancing early season field populations of *Eretmocerus* sp. using cantaloupe transplants. Cantaloupe seedlings prior to placement in fields were successfully inoculated with a highly specific whitefly parasite, *Eretmocerus* sp. recently imported from Ethiopia. We showed that fields receiving transplants inoculated with parasites (banker plants) have similar or greater parasitism of silverleaf whitefly than fields receiving conventional hand releases. Another goal was to determine whether this method could be integrated into conventional farming practices. We found that imidacloprid reduced parasitism in plots receiving transplants for silverleaf whitefly control.

In 1998 and 1999 we improved our methods for inoculating transplants with parasites. The number of parasites added to the fields increased from 9000 per ac in 1998 to 24,000 in 1999. During these years, we measured significant decreases on several sample dates in whitefly nymphal populations between plots receiving parasites and the no-release control plots. However, no consistent, significant differences were detected across both years when comparing insect population means between the two release strategies: hand versus banker plants. Parasitism means were consistently higher in the transplant plots in 1999, but about the same as in hand release plots in 1998.

In spring 2000 we showed that transplants significantly increased parasitism at 7 demonstration farms over the duration of the study, April through June, 2000 ($p=0.03$). Parasitism was higher in organic fields (seasonal average = 51%) than conventional ones (seasonal average = 10%). These data suggest that the imidacloprid treatment in conventional fields retarded the population growth of released parasites. Other factors such as poor survival of transplants or method of pesticide application, may also have contributed to poorer parasitism levels in conventional fields.

On average, the transplant treatment had no impact on whitefly populations at the 7 demonstration farms. Farms receiving transplants, whether organic or conventional, had on average the same silverleaf whitefly population density from April through June as the

paired controls (α=0.05). However, at some farms we found consistent, significant ($p<0.05$) suppression of whiteflies in treated plots over control.

The high levels of parasitism achieved in this study, plus suppression of whitefly numbers in replicated studies in 1998 and 1999, and in our paired-plot work of 2000 (two of seven fields), one organic and one conventional, show promise for this method. The banker plants provided at least the same if not better control of silverleaf whitefly than hand releases. Based on our own experience, the use of transplants as a means of adding parasites to a field would cost less than collecting parasites off plants and putting them into a field. However, more work needs to be done to make this strategy a predictable method. One area for research is the type of plants used. A tougher plant, capable of supporting more parasitized whiteflies deserves greater attention. Controlling a highly mobile pest such as silverleaf whitefly may be beyond the ability of augmentative biological control, at least in vegetables that have a very short growing season. Using transplants for augmenting natural enemies in other settings, such as greenhouses, perennial crops, or against pests with less propensity for rapid growth and dispersal, would have greater potential.

Introduction

The silverleaf whitefly (SLWF), *Bemisia argentifolii* (Homoptera: Aleyrodidae) Perring and Bellows, was a serious pest of several vegetable and field crops in desert agricultural areas in California, as well as other regions of the United States during the 1990s. Major crops affected in California include melons, cotton, cole crops, alfalfa, and tomatoes (Perring et al. 1993). During the early 1990's, silverleaf whitefly reached damaging levels on cotton and in some instances on citrus in the southern San Joaquin Valley, again raising fears that economically damaging populations of this pest were spreading to the more central regions of California's agricultural heartland. In the Imperial Valley alone, crop losses from 1991 to 1994 were estimated at 330 million dollars (Birdsall et al. 1995). Additional losses in sales and employment in this region have been estimated at 630 million dollars. Damage nationwide, was estimated at one billion dollars over this time (Faust & Coppedge 1995).

The regional impact of silverleaf whitefly in Imperial Valley on agriculture appears to have abated since the registration of imidacloprid (Admire™) in 1995 and changes in cropping patterns that disrupt continuity in whitefly host plants, i.e. the elimination of fall melon acreage. Most melon growers now use soil applications of imidacloprid that can provide 45-60 days of protection from whiteflies and other homopteran pests during spring months. However, given the silverleaf whitefly's capacity for rapid development of resistance to insecticides (Byrne et al. 1992, Prabhaker et al. 1992, Tan et al. 1996, Wolfenbarger & Riley 1994), and the demonstrated resistance to imidacloprid in laboratory studies (Prabhaker et al. 1995), other control strategies were investigated.

Recent studies in Imperial and San Joaquin Valleys show that early season releases of parasites can suppress populations of silverleaf whitefly. Simmons et al. (1995, 1996) increased parasitism levels over twofold in silverleaf infested spring melons in commercial fields when making early season releases of the exotic parasites

Eretmocerus spp. imported from India and Spain. Likewise, the density of whitefly nymphs was reduced by half. Similarly, Heinz et al. (1995) found a three fold reduction in silverleaf whitefly nymphs on cotton treated with releases of either *Eretmocerus* sp. imported from Texas or the native *Eretmocerus* from Imperial County. More importantly, augmentative releases may be used by growers treating cantaloupe with imidacloprid. In some fields, Simmons et al. 1996 released parasites following insecticide application, augmenting parasitism by twofold. Goolsby and Ciomperlik (1999) demonstrated in replicated laboratory studies that late instar *Eretmocerus* larvae can survive field recommended rates of imidacloprid used on whitefly infested cantaloupe. Early season releases of parasites would increase the regional population of silverleaf whitefly parasites and possibly reduce or eliminate the use of pyrethroids used later in the season for additional control of adult whiteflies. Increases in the regional population of the highly specific whitefly natural enemy *Eretmocerus* spp. would benefit summer and fall crops susceptible to silverleaf whitefly.

Just as with many other efforts at using augmentative releases of natural enemies in field or vegetable crops (Oatman 1970, Pickett & Gilstrap 1986, Pickett et al. 1987), the high cost of rearing the parasites needed for economic control of silverleaf whitefly may limit their use. In 0.5 acre plot studies, receiving ca. 40,000 parasites per acre *(Eretmocerus* ex. Pakistan), Simmons (unpubl. data) was able to maintain the same levels of parasitism in plots with and without applications of imidacloprid. He achieved similar levels of whitefly control when using imidacloprid plus releases of parasites as imidacloprid plus the pyrethroid bifenthrin. These results suggest that field applied imidacloprid has little if no detrimental affect on released parasites and that the same releases can achieve similar levels of control as provided by bifenthrin. The parasites would cost growers $200/ac who typically spend about $120 per acre for whitefly control. We are proposing that the cost of augmentation can be reduced further, and efficiency increased, by using transplants inoculated with parasites (banker plants). Rather than rearing whitefly and parasites on another non-crop plant, then harvesting and releasing the natural enemy, the crop itself is inoculated with parasites prior to its planting. Costs associated with growing non-crop plants are eliminated and mass rearing of parasites is reduced. Furthermore, fewer parasites introduced into a whitefly infested field on transplants may be needed to control whiteflies in a field receiving an equal number of hand released parasites. Hand released parasites must disperse over a large area, search for and then discover very low density prey while parasites emerging from inoculated plants would have prey readily available. The concept is simple, however no one has demonstrated the procedure from inoculation to field use and whitefly control. Large numbers of transplants previously infested with small numbers of whiteflies can be inoculated with parasites in a greenhouse setting. These plants could be mixed with conventional transplants or seeded fields at planting. Although not widely practiced, there is a growing trend to using transplanted melons, versus those that are seeded. In addition to inoculating plants with a highly effective natural enemy, advantages to growers include reduction in costs due to seed loss, and reduction in water and pesticide usage.

We report on results for the entire study, winter/spring 1998 to June 2000. During the first two years, a replicated study at one organic grower's field was conducted to compare the "banker plant" technique to a standard release method. Trials over the same period of time were also conducted in conventional fields to determine if transplants could be integrated into standard farming practices. In spring 2000 we demonstrated and measured the impact of the banker plant method at 7 different farm sites involving both organic and conventional growers.

Materials and Methods

Replicated, treatment comparison, 1998 and 1999.

A three treatment, replicated study was set up at the Bornt family's commercial organic farm in Imperial Valley in 1998 and 1999. Plots of cantaloupe receiving parasite-inoculated transplants were compared to plots with either "hand released" parasites or a no-release control. In 1998 plots measured about 1/3 acre in size in a ca. 5 acre field. The no-release control was assigned to the middle plot in each of 4 blocks, while the release treatments were assigned randomly to the outer of 3 plots. This was done to reduce movement of parasites between the two release treatments in such a small area. In winter/spring 2000 we increased the size of our plots to 1/2 ac., with treatments randomly assigned to each of 4 blocks. Plots were separated by at least one acre.

Transplants of the same or similar variety as to those in the commercial field were produced commercially at a private nursery in Brawley, California. Standard growing methods were employed for growing and maintaining plants through the entire study, less the use of insecticides. Transplant production began during the first half of January and continued until March, providing continuous plants of similar age for inoculation. Plants were moved to the USDA-ARS Field Station in Brawley 4 to 6 weeks after seeds were planted, and then inoculated with silverleaf whitefly in a closed greenhouse, free of other insects. Two weeks post inoculation, they were exposed to *Eretmocerus* adults produced in our own greenhouses on eggplant infested with silverleaf whitefly. *Eretmocerus* as pupae were placed in 50 ml. paper cups amongst flats of whitefly inoculated transplants in the greenhouse. Three to five days later flats were moved outdoors. For 10 days to 3 weeks plants were maintained in their original flats outdoors on tables, 1 m in height to harden them off for transplanting. Flats were watered daily.

The number of *Eretmocerus* used to inoculate plants was based on the number of whitefly nymphs on these plants. In 1998 a ratio of 1:1 nymphs to released parasites resulted in lower than expected pupal parasitoids developing on leaves and thus was changed to 10:1 in 1999 and 2000. Plants were placed in fields by hand between sunrise and noon. Ten percent of the original plants seeded by the grower, were replaced by our banker plants. Approximately every 10th seeded plant was removed and replaced with a banker plant, using a stick to produce a 3 inch hole. Holes were watered, plants added, then watered again minutes later, then as needed depending on the grower's irrigation schedule. One hundred plants in the center of each treatment plot were marked with a stick for future sampling. The grower's plants were of about the same age or up to two weeks older at time of transplant.

Plots receiving banker plants were visited weekly and the leaves of 100 staked plants centrally located in each plot examined for the presence of parasitoid exit holes. About five weeks after plants were added to plots, when exit holes were first detected, the leaves of 40 plants randomly selected from staked plants, were counted for number of *Eretmocerus* pupae and adult exit holes. This average was used to estimate the number of *Eretmocerus* released by hand. A portion of banker plants used on each planting was held back at the field station for six weeks. Numbers of parasitized whiteflies and parasite exit holes from these plants were compared against the staked plants to measure survivorship and actual number of parasites added to a field. Flats of these plants were maintained as above. Paper cups filled with *Eretmocerus* pupae and placed under the shading of plants constituted the "hand release" method. About 3 cups were placed down each row.

Paired comparisons: plots with and without banker plants

In winter/spring 1998 and 1999, in addition to the release treatment comparison, we also placed banker plants into conventional fields using imidacloprid for whitefly control. We wanted to measure the impact of banker plant produced parasites on silverleaf whitefly in fields using conventional farming practices. We were also interested in learning if imidacloprid affected population growth of released parasites. In both years two pairs of 1 acre each were delimited at each farm, a banker plant plot and control plot.

As with the above study, 10 % of the original melon plants seeded by the grower was replaced with one of our banker plants. Paired plots were separated by at least 1 acre. Plants were sampled for both silverleaf whitefly nymphs and released parasites, as above.

In winter/spring 2000, we set up paired comparisons in 7 commercial farms of cantaloupe in Imperial Valley to demonstrate, replicate and measure the impact of banker plants on parasite and whitefly populations across Imperial Valley: 3 organic and four using conventional practices, i.e. imidacloprid for whitefly control. At each farm two, 1 acre plots were delimited in a much larger field (20 acres or more), spaced at least 1 acre apart. In one of these plots, cantaloupe banker plants inoculated with *Eretmocerus nr. emiratus* (Ethiopia) were added to the field. Approximately 10% of the planted field was replaced by our banker plants, as above. The survivorship of parasitized whiteflies on banker plants was compared between plots in organic and conventional fields by randomly selecting 40 plants from 100 numbered, staked plants centrally located at each study site, as above. These were sampled and examined 5 weeks after planting for the number of parasite exit holes and pupae. These values were also compared against survivorship of parasitized whiteflies on banker plants held back and maintained at the experiment station.

Results and Discussion

Replicated Field trials comparing release systems: Field Season 1998 and 1999

The number of parasites added to the replicated trial at the organic farm increased from 9000 per ac in 1998 to 24,000 in 1999. In both years we measured significant differences on several sample dates in whitefly nymphal populations between the different treatment plots (Figs. 1, 2). Both years, the whitefly numbers were lowest in the plots receiving parasites. However, no consistent, significant differences were detected across both years when comparing insect population means between the two release strategies: hand versus banker plants. Parasitism means were consistently higher in the banker plant plots in 1999, but about the same as in hand release plots in 1998. Plots were smaller (1/3 ac) and much closer to each other in 1998, promoting cross contamination among treatments. This was not the case in 1999. Whitefly numbers were generally lowest in the banker plant plots in 1998, but mixed in 1999.

Paired comparisons: plots with and without banker plants.

Field season 1998/1999. No consistent results were observed in the conventional fields receiving banker plants in terms of increased parasitism and whitefly control. In 1998 parasitism and whitefly numbers were about the same in both control and treated plots (Fig. 3). Parasitism was much higher in the treated plots than in the no-release control at Black Dog Farms in 1999. In this year we added banker plants to three farms. However at only the Black Dog farm did our plants survive. Gaps between irrigation scheduling and heavy winds killed our plants at the Strahm and Abatti farms by mid season. Levels of parasitism in both of these years in the conventional plots receiving banker plants were consistently lower than at the organic farm used in the treatment comparison. Parasitism in the conventional fields receiving banker plants averaged 2.1% in 1998 and 7.5% in 1999. Parasitism in plots receiving banker plants at the Bornt's organic farm with the replicated trial averaged 18.0% in 1998 and 28.5% in 1999.

Demonstration field trial 2000. In winter/spring 2000 we increased the average number of parasites per banker plant up to 25, or about 25,000 per acre: range 5,000 to 45,000. No relationship is visually apparent between numbers released in a field and seasonal parasitism or whitefly densities (Table 1, Figs. 4 and 5). A repeated measures ANOVA, blocking for type of grower (organic vs. conventional) showed that banker plants significantly increased parasitism in study sites over the duration of the study, April through Jue, 2000 ($p=0.03$). Parasitism was higher in organic fields (seasonal average = 51%) than conventional ones (seasonal average = 10%). The average number of parasites per banker plant was numerically higher on the banker plants in organic sites compared to conventional plots after 5 weeks, but was not significantly different (32 vs. 21 per plant; $p>0.05$; Table 1)**.** These data suggest that the imidacloprid treatment in conventional fields retarded the population growth of released parasites. Other factors such as poor transplant survival, may also have contributed to poorer parasitism levels in conventional fields.

A repeated measures ANOVA showed that banker plants, on average, had no impact on whitefly populations. Plots receiving banker plants, whether organic or conventional, had the same silverleaf whitefly population density from April through June as the paired controls ($\alpha=0.05$). However, some study sites showed a consistent suppression of whiteflies in treated plots over control plots (Fig. 4 and 5, e.g. Bornt's organic field #2 and Strahm's conventional field. Although one of the Bornt plots achieved very high levels of parasitism (#1, near his house), the releases had no net affect on his silverleaf whitefly populations. This may have occurred because the study site was within 200 m of a hedge planted specifically for promoting the overwintering survivorship of whitefly parasites. Results from Heger's field are more difficult to explain, since the banker plant treated plot had consistently high levels of parasitism, yet no measurable impact on the whitefly population. Silverleaf whitefly are known for their ability to migrate long distances. We and others have measured their ability to disperse, which is one plausible explanation. The imidacloprid treatment in the two conventional fields Abbatti 30 and 182 probably had a greater impact on the whitefly population than did the banker plants. We did not monitor exactly how and when applications of this systemic were applied. Timing and rates of application could easily have caused between site differences in our results.

The high levels of parasitism achieved in this study, plus suppression of whitefly numbers in replicated studies in 1998 and 1999, and in our paired-plot work of 2000 (two of seven fields), one organic and one conventional, show promise for this method. However, more work needs to be done to make this strategy a more predictable method. One area for research is the type of plants used. A tougher plant, capable of supporting more parasitized whiteflies deserves greater attention. Controlling a highly mobile pest such as silverleaf whitefly may be beyond the ability of augmentative biological control, at least in vegetables that have a very quick growing season. Using banker plants for augmenting natural enemies in other settings, such as greenhouses, perennial crops, or against pests with less propensity for rapid growth and dispersal would have greater potential.

The work also shows that imidacloprid retarded the growth rate of the released parasites. In some instances, the combination of both parasites and this insecticide reduced silverleaf whitefly over that of fields not receiving banker plants. It's possible that some conventional fields used a higher dosage than others or the insecticide was used more frequently, e.g. more than one application. Such differences could have affected the performance of released parasites.

Conclusions

This study demonstrates for the first time augmentative biological control of a vegetable pest using transplants. Parasitism levels were increased several fold in both organic and conventionally produced melons using parasite inoculated transplants (banker plants) placed in fields during the first few weeks of the growing season. Results will be published in a peer reviewed journal. Banker plants could also be used in greenhouse settings, other vegetable crops using transplants and even in field crops. Methods were developed to inoculate plants, parasite populations were augmented over natural levels, and in instances, whitefly populations were significantly reduced in both organic and conventional fields. Transplants proved at least as effective at augmenting *Eretmocerus* spp. as when released by hand. Our experience suggests that transplants would cost much less than releasing parasites by hand or using equipment. Removing adults or pupae from plants is labor expensive.

Our study also showed that banker plants could not consistently reduce whitefly numbers. Problems associated with whitefly migration and use of insecticides most likely confounded our results. One consistent result was that levels of parasitism in conventional treated plots was lower compared to organic treated plots. Although other studies have found no impact by imidacloprid on releases of *Eretmocerus* spp. (Goolsby and Ciomperlik 1999, Simmons, unpubl.. data), the placement of banker plants into treated fields appears to place the developing pupae on these plants in close proximity to this systemic toxin.

The use of a tougher plant could greatly improve the performance of banker plants and is a ripe area for additional research. Cantaloupe proved vulnerable to water stress. Species of *Brassica*, i.e. broccolli, would support many more pupae, withstand transplanting far better and could perhaps be harvested. Furthermore, a species of plant other than the one being grown would indicate to the grower which plants were inoculated with parasites.

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Fig. 1. Organic melons, Imperial Valley, 1998.
Effect of different release methods. Means ± 1 SE.

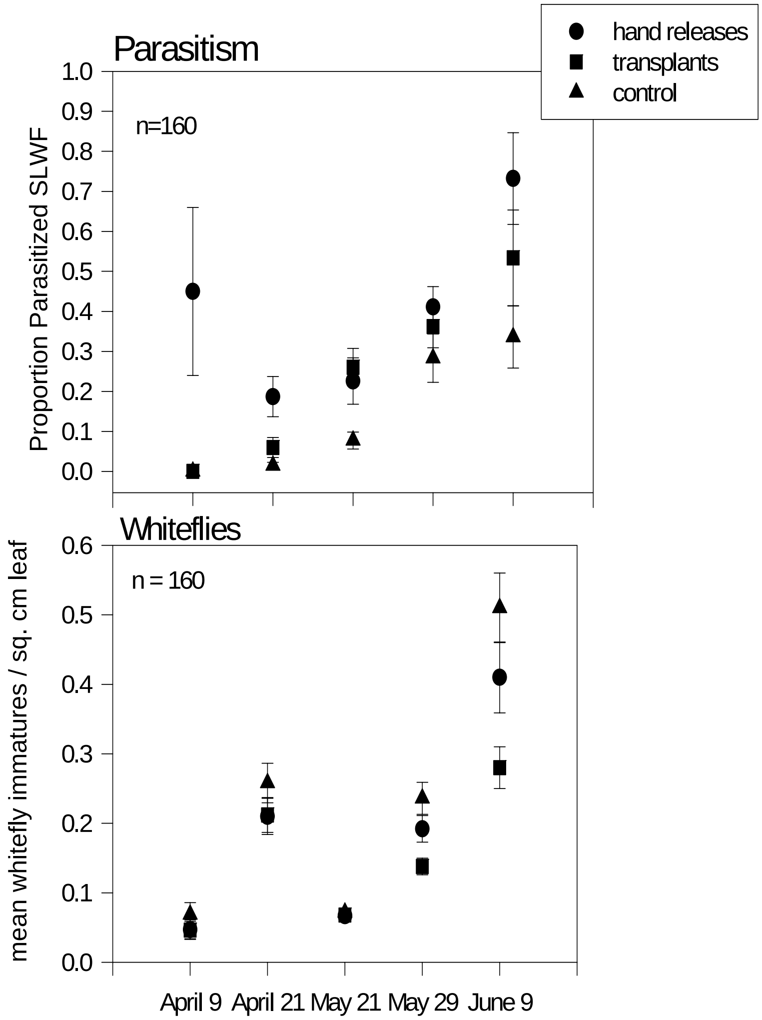
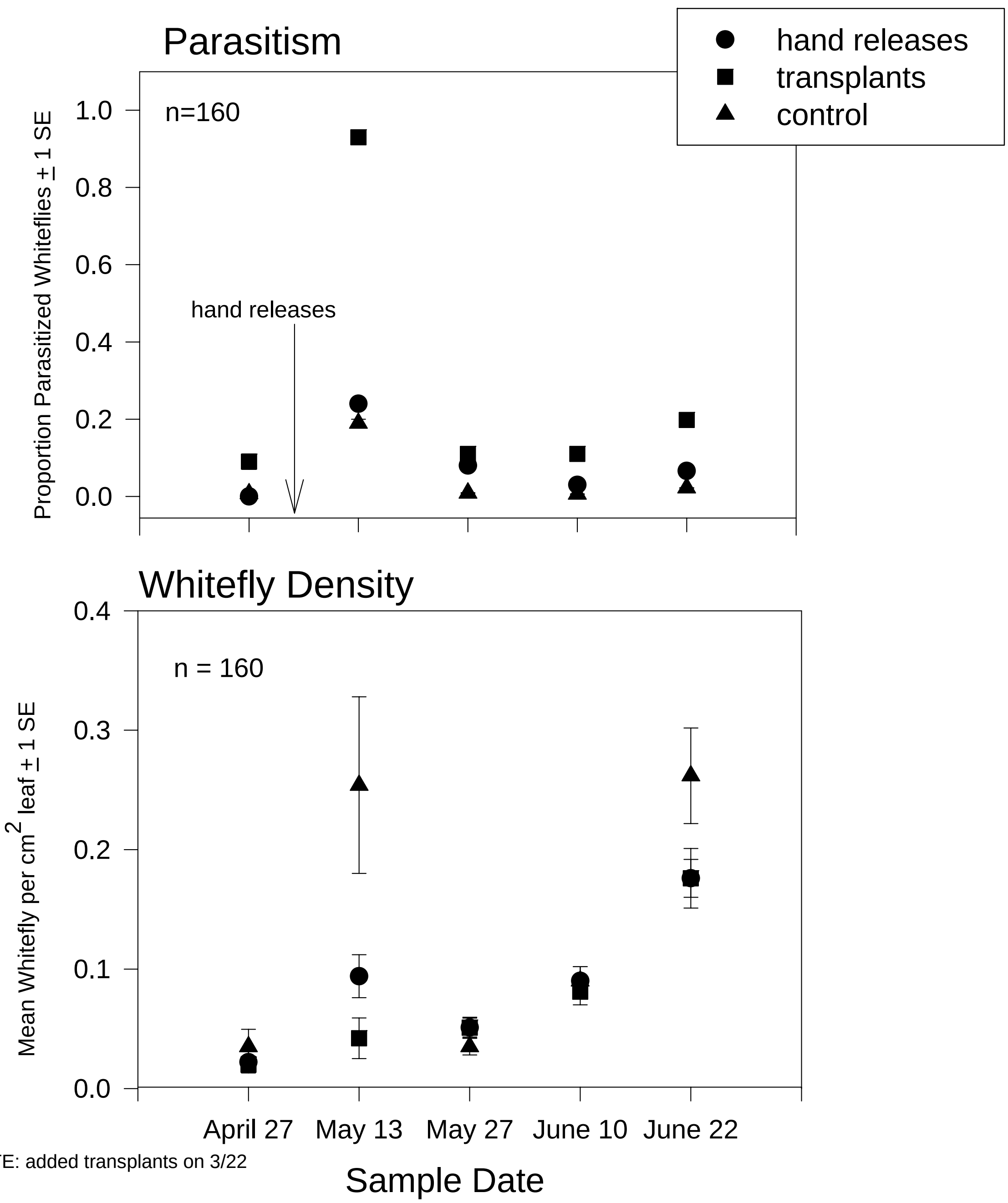


Fig. 2. Organic Melons, Imperial Valley, 1999.
Effect of Different Release Methods.



NOTE: added transplants on 3/22

Fig 3. Releases into Conventional Fields 1998 and 1999.

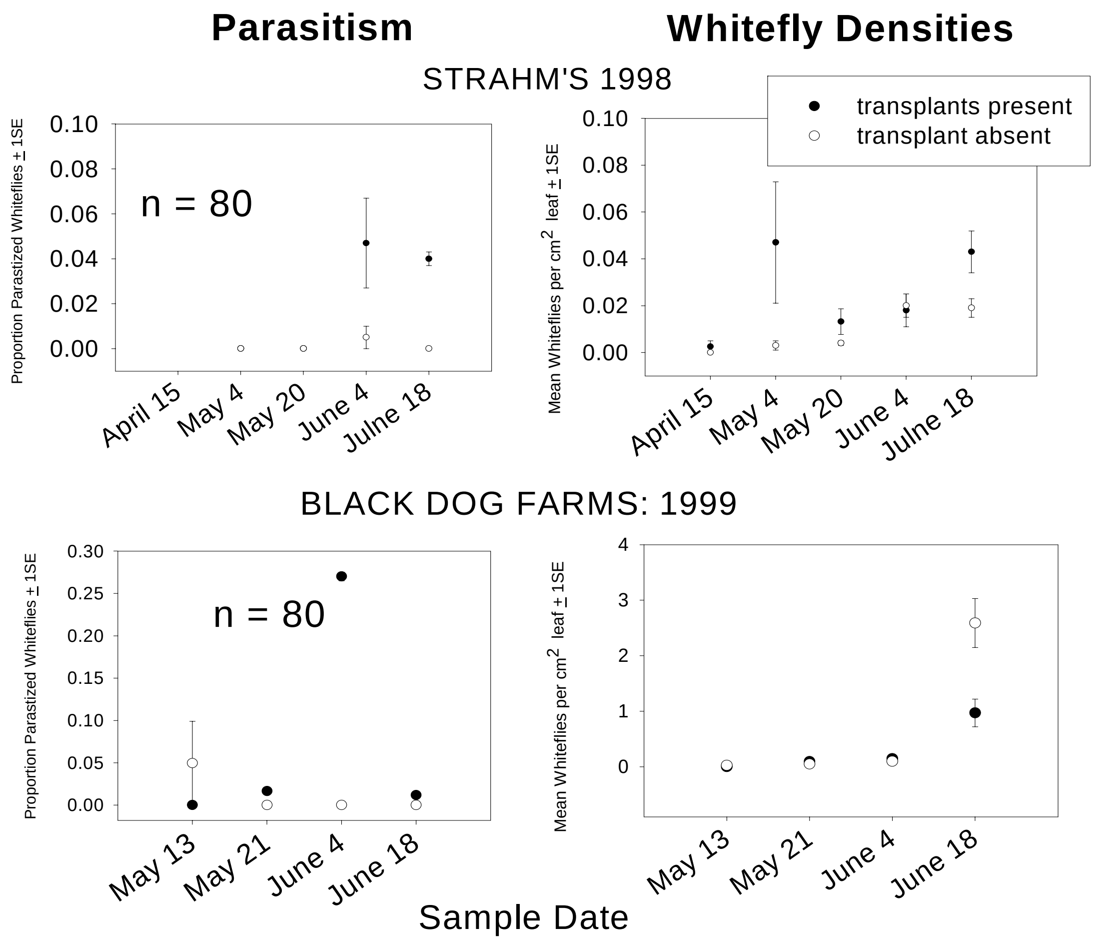


Fig. 4. Organic plots receiving banker plants. Imperial Valley, spring 2000.

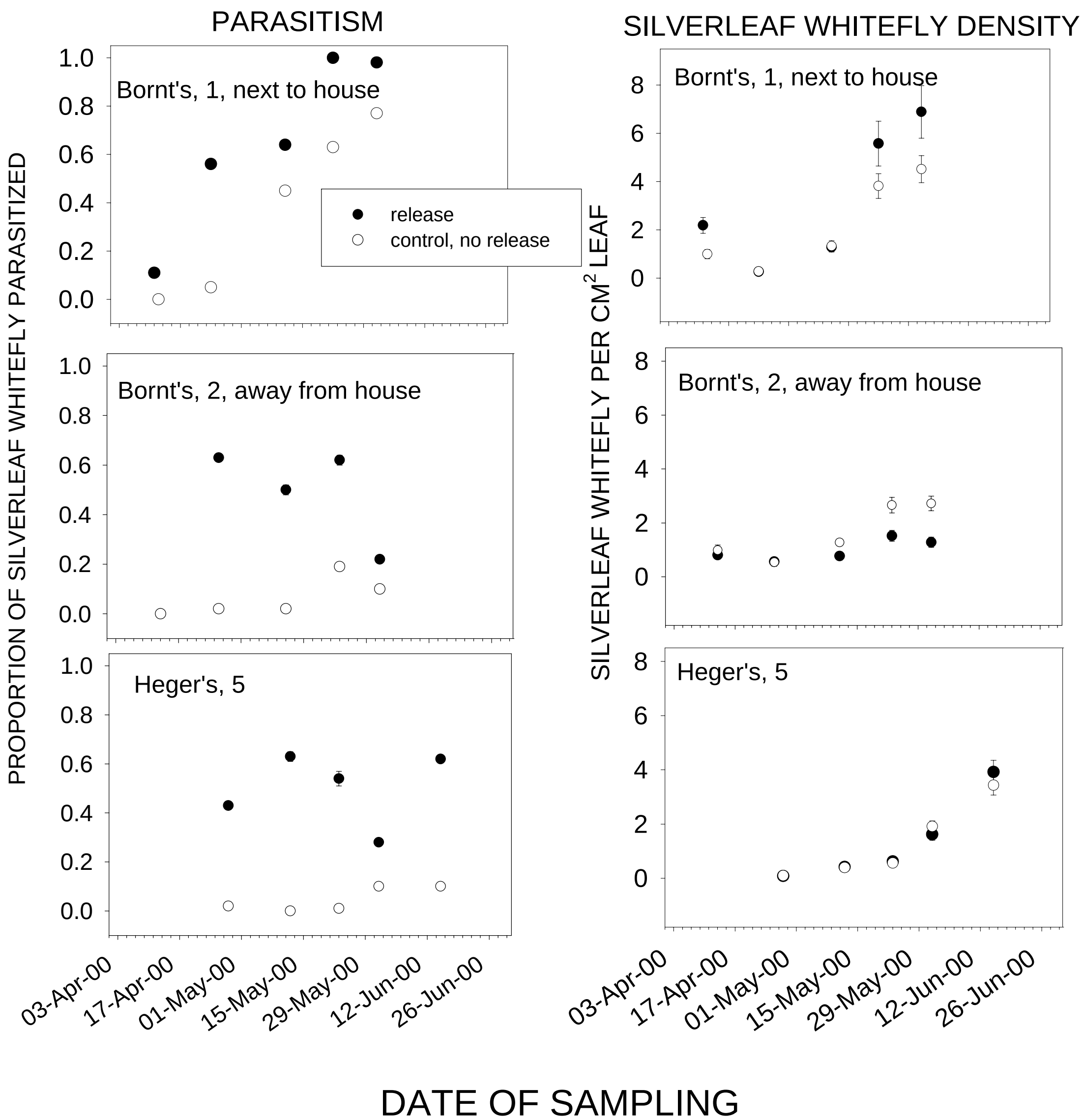


Fig. 5. Conventional plots: treated with both banker plants and imidacloprid.

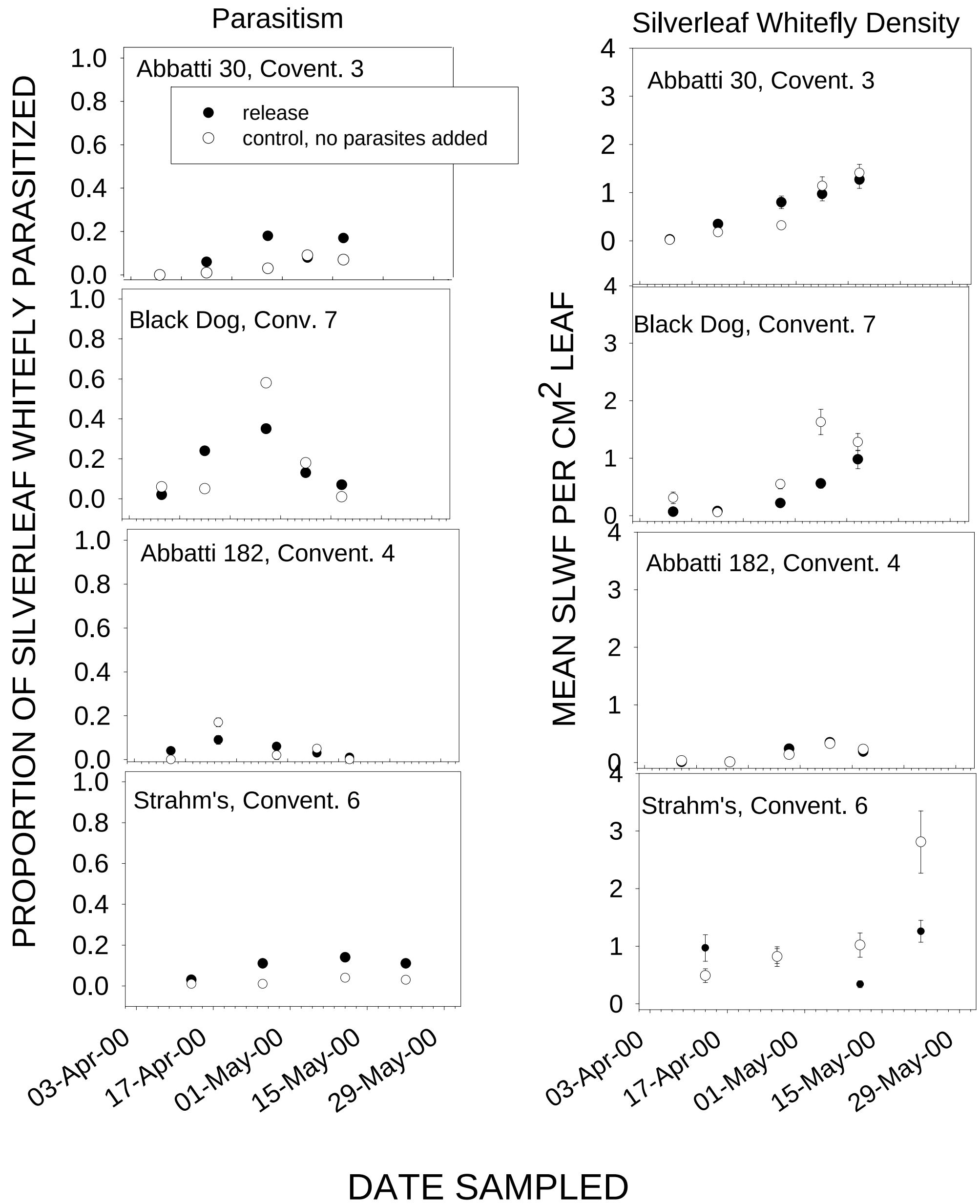


Table 1. Grower in spring 2000 and mean insects per plant 5 weeks post-planting.

Field Name	type of grower	number	mean Eretmocerus	mean SLWF
Bornt's near house	organic	1	20.3	1.5
Bornt's away from house	organic	2	45.3	1.5
Abbatti 30	conventional	3	5.02	0.48
Abbatti 182	conventional	4	40.6	1.96
Heger	organic	5	32.1	1.55
Strahm	conventional	6	7.1	2.42
Black Dog	conventional	7	29.8	1.56